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Silicon nanotweezers for biomechanical and bioelectrical assays

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1. ABSTRACT

In modern life, technologies enabling detection of biological molecules at a low threshold, for health and ecological concerns, are in high demand. Directly interrogating the molecules is a promising direction to clarify the noisy response of conventional assays arising from simultaneous different reactions. Besides sophisticated biophysical instrument such as the atomic force microscope, this paper proposes silicon nanotweezers (SNT) as a new microsystem for molecular manipulation. SNT can trap molecules and sense their biomechanical and bioelectrical response in minute operations. In this review SNT characteristics are overviewed; their operation modes are illustrated by molecule and cell trapping, manipulation and characterization in air and in solution. As they are tiny and can be mass produced by highly parallel microsystem technology, SNT can be seen as a potential molecular and cellular probe for routine analysis and bio detection.

2. INTRODUCTION

Nowadays, convenient methodology to detect tiny level of specific agents in biological or environmental samples is in high demand. Many technological approaches are flourishing to address such health and ecological concerns. Aside from approaches using high-throughput analyses of thousands of assays at the same time (1), technologies enabling analysis in tiny volumes are excellent candidates; analysis response can be short with reduced amounts of costly reactants. Such a microfluidic approach (2) permits biological diagnostics (3, 4) and drug screening (5). Even so, the analysis is performed in a tiny chamber, many reactions simultaneously occur and the read-out remains noisy and the result uncertain. To clear away this uncertainty, the detection has to be performed at even the smaller scale by directly interrogating the molecules (6).

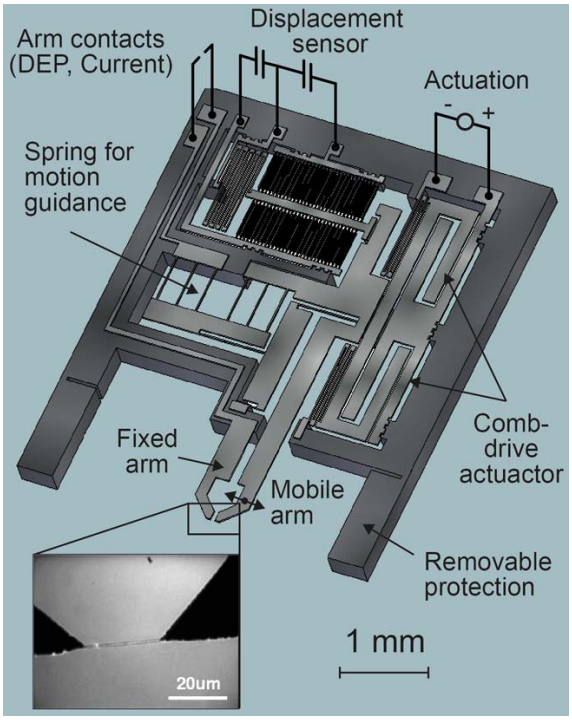

Figure 1. Comprehensive view of the silicon nanotweezers indicating the different parts and their functionalities. The input/output ports are indicated. The inserted picture is a closer view of DNA bundle captured between the opposing sharp tips.

The most direct proven way consists in trapping the molecule and in sensing its biomechanical response when exposed to reacting agents. This molecular manipulation has already revealed fundamental information on the physical properties of biological systems (7), for understanding the mechanism behind essential enzymatic reaction like DNA wrapping (8) and replication (9, 10) and more recently virus dynamics (7, 11).

These experiments are routinely performed either with optical tweezers (12, 13), magnetic tweezers (14, 15) or atomic force microscopes (16, 17) (AFM). These biophysical instruments are sophisticated, rather expensive, require experienced manipulation skills and controlled environments. Moreover, the molecules have to be prepared one at a time, to anchor them on cover glass or to bind functionalized microbeads at their extremities for their optical or magnetic manipulation. These constraints hardly fit with routine bio sensing operations.

Nevertheless, the achievements of AFM in molecular biophysics incited us to exploit further the possibilities offered by silicon micromachining technology; MEMS devices can integrate accurate molecular-level engineering tools (18), are compatible with micro fluidics (19) and are cheaply produced using parallel processes. The concept of silicon nanotweezers (SNT) arose from successful minute trapping of DNA bundle between micro fabricated opposing sharp tips (20). Thus further integration of actuation and sensing to this trapping capability, provides SNT with mechanical manipulation, and electrical sensing at the molecular level.

In this paper, SNT is presented as a generic micro device allowing biomechanical, bioelectrical assays of filamentary molecules and cells. SNT characteristics are overviewed; their operation modes are illustrated by molecules trapping, manipulation and characterization in air and in solution.

3. DESCRIPTION OF SILICON NANO TWEEZERS (SNT)

The huge progress of single molecule manipulations has clarified the functionalities required for routine molecular-level bio-assays and analysis. The molecules have to be trapped with minute procedure and delivered into the diverse solutions and environments in which the targeted reaction will occur. The reaction is detected by the direct physical stimulation of the molecules, either by mechanical or electrical means, and the response of the systems has to be sensed in real time, via the modification of the stiffness of the molecule or changes in its conductivity. The development of the protocol needs a visual control, that will be replaced by an automatic monitoring once suitable protocols have been established. All these requirements have been integrated into a unique MEMS device, referred to as the Silicon Nano Tweezers (SNT) and its experimental setup. The SNT (Figure 1), is made by silicon processing and its size is less than six mm by six mm (21). The SNT consist of two arms ending in sharp opposing tips between which the molecules are captured and manipulated. These tips are electrically connected to the arm contacts and act as electrodes to trap the molecules by Dielectrophoresis (DEP) and to measure their conductivity. One arm is fixed, the other one is mobile and is displaced by an electrostatic actuator. This gap between the tips is tuned by the voltage difference applied between the two actuation voltage ports. The displacement of this arm, corresponding to the elongation of the molecules is measured by a capacitive position sensor. As the tips aim to be immersed in biological solutions, the SNT design has the sensor on the other extremity of the arm so as to protect it from any contact with the liquid. Thus, a guiding mechanism inspired from high precision linear scanner has been implemented to ensure a proper translational motion of the arm with reduced rotation (22). The tweezers are processed by standard micromachining from Silicon on Insulator (SOI) wafer. The active and mobile parts are patterned in the upper silicon layer (25 µm thick), whereas the hard frame of the device is machined in the thick silicon bulk (550 µm thick). This technology allows mechanical linking of the movable parts, while keeping them electrically insulated. The shape of the opposing tips and the gap between them are tuned by mask design and etching procedures. At the end of process, a 50 nm thick aluminum layer is evaporated on the front side. This Al layer acts as an anchoring material for DNA (23) and other biomolecules and improves the sheet conductivity of SNT parts and the electrical output noise of the sensor.

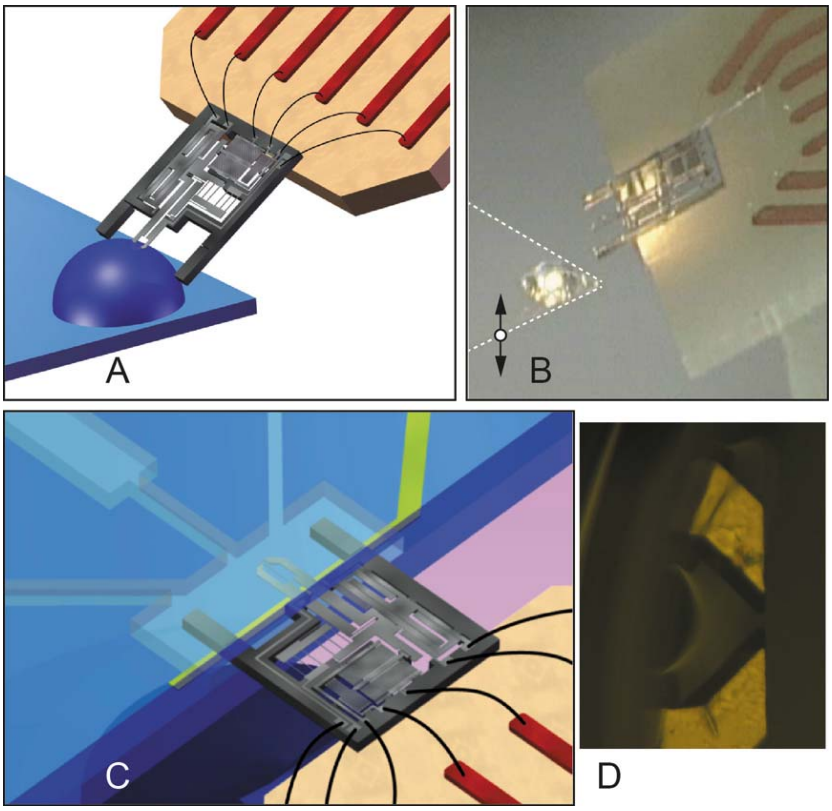

Figure 2. Operation of SNT in solution. A: Schematic view of "in droplet" operation. B: Corresponding close-up view. C: Illustration of the tweezers in a microfluidic cavity, for "in slit" operation and, D: close up photograph of the opposing tip through the PDMS layer.

4. OPERATION IN SOLUTION AND TRAPPING OF FILAMENTARY MOLECULES

The operation of solid-state Micro Electro Mechanical Systems (MEMS) in solution requires an appropriate treatment of the air-liquid interface effects. A complete immersion of the device, as it is the case of the cantilevers of liquid AFM, allows removal of interfacial forces such as the meniscus effect and the surface tension but requires specific assembly. The actuator has to be hermetically sealed, the cantilever needs to operate in a closed microfluidic chamber and its motion is remotely captured by an optical readout. This strategy hardy complies with the integrated actuation and sensing of the SNT for several reasons. Once polarized, the conductive immersed parts of the tweezers will initiate electrolysis reactions incompatible the integrity of biological systems. The tweezers can be capped by thin isolating layers to avoid these electrochemical reactions. This process is indeed feasible with micro-fabrication techniques (24) but the free standing structures would be significantly deformed by the bimorph effect of the silicon/isolator bilayer. The electrostatic actuation in conductive solutions is also a challenging issue as the electric field between the 2 motional electrodes is screened by the counter ions of the diffuse double layer (DDL) (25). Thus, the actuation needs a high-frequency modulation of the actuation signal to block DDL formation (25), but signal coupling generates excessive electrical noise in the displacement sensing and molecular resolution is lost. Completely immersed, the tweezers will experience important viscous drag that would degrade its motional characteristics such as its quality factor when put in the resonance mode. Finally, the size of the fluidic chamber would be too cumbersome to fit the microsystem concept.

Thus, to keep the advantages of the tweezers integration and performance, they are operated in air, and only the tips are immersed in the solutions (Figure 2). Two configurations are currently used. One corresponds to operation in droplets (Figure 2A and 2B). In the second configuration (Figure 2C and 2D), the tips are inserted in a lateral slit of a microfluidic chamber. This latter setup allows us to change the liquid medium with appropriate microfluidic operation. The cavity is formed by a structured PDMS layer, obtained by molding (26) and reported on a glass cover slide. The slit and the microfluidic cavity are 200 µm in height.

The influence of the air / liquid interface on the biomechanical measurements of the immersed sample, is minimized in two ways. First with the device design, the arms are only 25 µm in thickness while their spacing at the meniscus location is larger than 150 µm (Figure 2D), this aspect ratio favorably reduces the intensity of the capillary forces acting between these two parts. Second, this reduced

Table 1. Bio sensing capabilities with SNT

Samples	Air or Vacuum	Solution
Molecules	Elect[1], Mech[2]	Mech[3]
Nanowires	Elect[1], Mech[2]	Elect[4], Mech[3]
Cells	None	Mech[5]

[1]Current measurement precision: 2 pA. (Under 10V, resistance up to 5 TΩ) [2] Maximum displacement 3 µm, resolution 0.2 nm. In static mode the force resolution is 10 nN, [3] With the SNT put in resonance, the force gradient (stiffness) precision is 0.5 $mN.m^{-1}$, [4] The resistance measured by bare tweezers is deionized water is 20 MΩ for 10 µm gap. Lower resistive nanowires can be measured, [5] Mechanical measurements consist in compressing the cell and to measure its restoring dynamics.

interfacial force is kept constant during the measurements by the stabilization of the meniscus position. In a droplet, the chosen deposited volume is large enough (1 µl) to neglect the evaporation effect on the meniscus location, this configuration is suitable for quick operations. In the microfluidic operation, the tiny slit aperture grasps the meniscus at a fixed location and long time stability is routinely achieved. Thus, these interface forces are offset, with constant values that are then canceled out from the measurements.

DNA trapping is an appropriate illustration of SNT operation in liquid, the procedure is here detailed for the "in droplet" configuration. The trapping of molecules bundles by DEP with the silicon tweezers follows the technique developed by Hashiguchi *et al.* (20), inspired from pioneering experiments by Washizu *et al.* (27). A small droplet of DNA solution (Figure 2A) is first deposited on a microscope cover slip. (48502 bp λ-DNA Takara Bio Inc., Shiga, Japan diluted 1:1 with DI water). The tweezers, mounted on a printed circuit board is maintained at a fixed position. The tweezers tips are smoothly brought into contact with the surface of the droplet by moving the coverslip upwards with a three-dimensional (3D) mechanical stage (Figure 2B). When the tips are immersed, but still forming a meniscus, an AC electric field (1 MHz, 1V/µm) is applied between the two arms of the tweezers. Due to the intense electric field, DNA molecules are stretched and attracted towards the tips and finally form a bundle between them. More detailed explanation of the mechanism of DNA stretching and trapping by DEP and its theoretical background can be found in Washizu *et al.* (28). The diameter of the bundle varies by DEP time, and can range from several 10 nm (10 s DEP) to ≥ 300 nm (5 min. DEP). Even single DNA molecule can be trapped with DEP with very short pulses (5 ms) (29).

The strong DNA binding to the aluminum-coated tips prevents the bundle from being washed away due to the surface tension of the solution. The DNA bundle is then accessible for visualization under either an optical microscope, or using a Scanning Electron Microscope, (insert of Figure 1) and for mechanical (22) and electrical characterizations (30).

5. BIOSENSING CAPABILITIES

The MEMS concept, summarized by the integration of grippers, actuator and sensor in a tiny device, provides the SNT with a wide range of bio-sensing capabilities. The actuation can stretch or compress the trapped sample, while the displacement sensor measures its response in real time enabling its mechanical characterization. The stimulation can be static, step-like (step response), or a harmonic (frequency response). These analyses allow study of the rheology of the samples by identifying its basic mechanical components such as mass, stiffness and viscosity (losses) (31). The actuation, sensing electrodes and the tips are mechanically linked but electrically isolated, the conductivity of the sample can be measured at rest or under strained conditions. The manipulation and characterization can be performed in ambient condition, in vacuum and with tips immersed in solution. The tips can be appropriately designed to trap filamentary molecules, nanowires or cells. All these features represent numerous bio sensing capabilities that are summarized, for sake of a comprehensive survey, in Table 1. Also mentioned are the resolution or the precision for each type of measurement, for the mechanical sensing, these data have to be compared to single base pair pitch of B-DNA, 0.34 nm, the stiffness of λ-DNA in its elastic regime 66 µN/m and the force needed to alter its double helix (B-DNA) structure, 65 pN (12).

6. BIOMECHANICAL CHARACTERIZATION OF DNA BUNDLES IN AIR

The characterization method with SNT is illustrated by the measurement of the mechanical properties of DNA in air. After the DEP trapping (Figure 1A), once retrieved from the solution, the DNA bundle is ready for analysis in ambient condition. The mechanical characterization is carried out by the frequency response of the SNT holding the bundle. Sinusoidal voltage of constant amplitude and frequency scanning (step by step frequency increase) is supplied to the actuator and the capacitive current of the displacement sensor is recorded. With the bundle trapped between the 2 tips, the resonance of the tweezers shifts to higher frequencies due to the added stiffness brought by the bundle (Figure 3). At the same time, the quality factor of the resonance is degraded, the maximum displacement is lowered and the peak extends to a wider frequency range, this degradation indicates the presence of viscous losses in the DNA bundle during its deformation. Therefore, the SNT behaves as a damped oscillator consisting of an oscillating mass attached to a mechanical spring and a damper. Using this damped oscillator model, the resonance frequency and the quality factor are extracted by least squares optimization (31). For the tweezers alone and with two different attached bundles, the model matches the measurements with a remarkable accuracy (Figure 3A). As the tweezers and bundle undergo the same deformation, their mechanical components add up (Figure 3B) and the total stiffness and viscosity are calculated from the resonance frequency and quality factor of the oscillating system. The bundle characteristics are then deduced by subtracting the bare tweezers contribution from the total stiffness and viscosity. The resonance frequency and quality factor extracted from the 3 experiments (Figure 3A) are given in the Table 2. The calculated stiffness and viscosity of the SNT and the 2

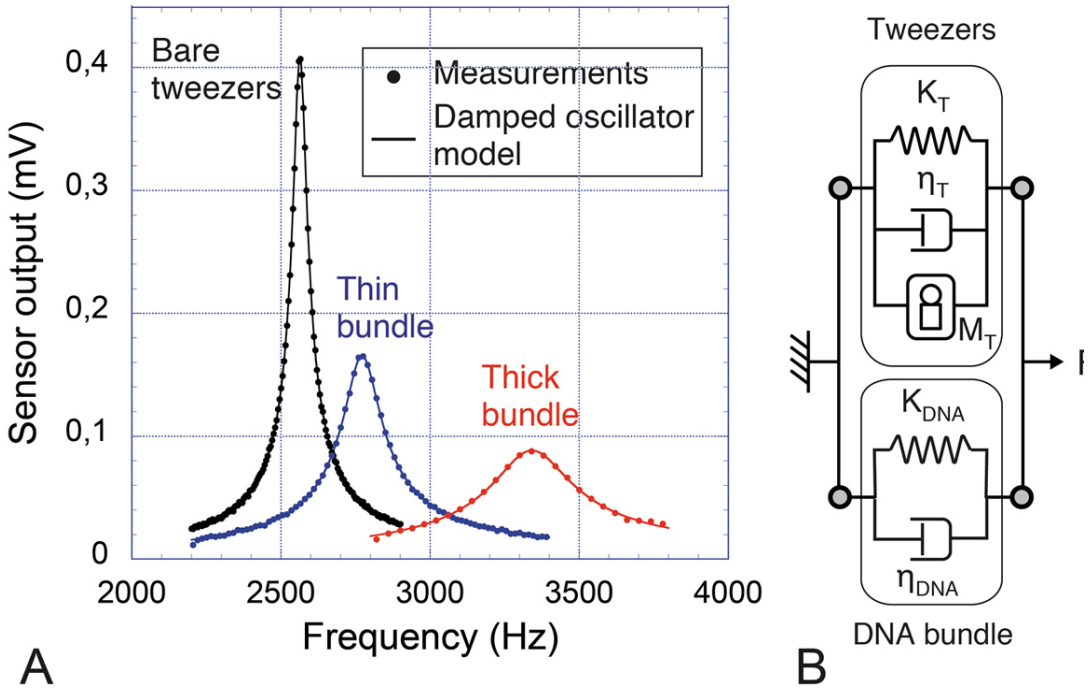

Figure 3. A: Frequency responses of the tweezers for the characterization of the tweezers alone and with 2 different trapped bundles. For each curve, the best fitted damped oscillator model is superimposed on experimental data. B: Dumped oscillator model with basic components for tweezers and bundle.

trapped bundles are also mentioned with their relaxation time. The precise extraction of the frequency determines the bundle stiffness with a resolution of 0.5 $mN.m^{-1}$ noted in the Table 1. This precision is suitable to sense tiny structural alterations of the bundle and thus provide the tweezers with ambient sensing capabilities.

7. BIOMECHANICAL CHARACTERIZATION OF GELATIN FILAMENT IN SOLUTION

For more relevant biological applications, the SNT have to measure the biomechanical response of molecules in liquid. This ability is demonstrated by the characterization of gelatin filaments in solution just after their trapping. Gelatin solution is prepared with diluting a commercial product (Jellice Maruha, Japan) with DI water to a mass concentration of 85 mg/L corresponding to an averaged concentration of 200 nmol/L. A 5µl droplet of the gelatin solution is deposited on the corner of a cover glass and SNT tips are immersed with the same protocol as for DNA trapping (Figure 2B). Depending on the dielectric properties of biomolecules and medium, the DEP conditions have been tuned even on a wide range to obtain correct trapping yields (32). Here, after numerous unsuccessful trials with 1MHz DEP signal (the appropriate frequency for DNA), gelatin filaments were found to be efficiently trapped by increasing the frequency at 5MHz while keeping the electric field at 1MV/m between the tips (40 V_{pp} for 20 µm gap). The mechanical resonance characteristics of the SNT were recorded in air and with tips immersed in the solution before and after the gelatin trapping. These responses are super-imposed in Figure 4 and their evolution can be comprehensively analyzed. When put in immersion, a resonator sees its effective mass increased by the fluid boundary brought in motion by its vibration (33), its resonance frequency decreases. The resonator also experiences a reduction of its quality factor due to the significant viscous damping (34) in liquid. Even if only the tips of the SNT are inserted in the solution, these effects are obviously observed by comparing the bare tweezers characteristics before (curve 1) and after (curve 2) the immersion. After the DEP application during 1 min., the resonance frequency re-increases while the Q factor experiences an additional decrease with the stiffness and viscosity brought by the trapped gelatin fiber. These evolutions between the curves 2 and 3 are similar to that was observed in Figure 3 for DNA in air. Thus using accurate extraction with the damped oscillator model, the mass of the fluid boundary can be calculated as the gelatin fiber characteristic, these data are given in Table 3.

Gelatin nanofiber clusters act as an interesting material as they combine both the high surface/volume ratio of the nano structuration and the versatile properties of polymer material. Many applications are foreseen such as in filtration, sensing and biomedicine (35) for which the fibers form an appropriate substrate for cell cultures (36). The polymer preparation needs to be tuned to reach the application-specific properties. Thus, the manipulation of the fiber in a microfluidic cavity with different inputs, as illustrated in Figure 2C is a versatile way to test different additive to reach the targeted properties. Indeed, the same approach consisting to sense in real time the response of biomolecules, such as DNA (7) or microtubule (37) is even

Table 2. Mechanical characteristics of tweezers and DNA bundles

	SNT	Bundle 1	Bundle 2
M (Kg)	$1.9\ 10^{-7}$ (1)	ε (2)	ε (2)
F (Hz)	2563.42	2771.67	3341.11
Q	55.83	22.65	13.01
K ($N.m^{-1}$)	49.3 (3)	8.30 (4)	34.4 (4)
η ($N.s.m^{-1}$)	$5.49\ 10^{-5}$ (3)	$9.11\ 10^{-5}$ (5)	$2.52\ 10^{-4}$ (5)
T (s) (6)	$1.11\ 10^{-6}$	$1.10\ 10^{-5}$	$7.32\ 10^{-6}$

(1) SNT mass is calculated from processed device dimensions, (2) DNA bundle mass is negligible, (3) Truncated value, (4) Bundle alone stiffness, truncated value, (5) Bundle alone viscosity, truncated value,(6) Relaxation time: η/K

Table 3. Mechanical characteristics of tweezers in air and in solution without and with gelatin filament

	SNT in air	SNT in sol.	Gelatin
M (Kg)	$1.9\ 10^{-7}$ (1)	$1.2\ 10^{-9}$ (2)	ε (3)
F (Hz)	2780.13	2771.67	2775.25
Q	65.18	49.01	34.01
K ($N.m^{-1}$)	58.0 (4)	58.0 (4)	0.157 (5)
η ($N.s.m^{-1}$)	$5.09\ 10^{-5}$ (4)	$6.79\ 10^{-5}$ (4)	$2.99\ 10^{-5}$ (6)
T (s) (7)	$8.78\ 10^{-7}$	$1.17\ 10^{-6}$	$1.90\ 10^{-4}$

(1) SNT mass is calculated from processed device dimension, (2) The mass of the fluid boundary, (3) Gelatin filament mass is negligible, (4) Truncated value, (5) Gelatin alone stiffness, truncated value, (6) Gelatin alone viscosity, truncated value, (7) Relaxation time: η/K

more attractive. The real time measurement of their biomechanical changes allows monitoring of enzymatic or chemical reactions for bio sensing or drug discovery (19).

8. CELL COMPRESSION ASSAY

Biological cells adapt their structures to respond to the local environment by generating forces from internal chemical to mechanical energy conversion. This conversion relies on numerous enzymatic reaction triggered by complex regulation scheme revealing the cell condition. The reciprocal link between the mechanical properties of the cell in its development and in pathologies is now well accepted. For example, invasive cancer cell lines exhibit rheological properties distinct from their noninvasive counterparts, (38) and change in cell properties can induce cancerous behavior (39). Thus biomechanical characterization proves to be relevant for cell bio sensing (40) and drug screening (41). Numerous technics have been developed and applied for measuring individual cell mechanical properties (42), such as aspiration by pipet, indentation by AFM, stretching by optical and magnetic tweezers. However, for routine assay, SNT provide a label-free alternative as the cell can be directly trapped, manipulated and deformed between the 2 tips.

The SNT fabrication process was modified to yield arms terminated by a pair of flat tips (Figure 5A). The gap between the 2 surfaces was designed to be 12 µm, close to the diameter of the fibroblast cell under study. The actuator linked to the mobile arm was adapted to close the gap to stimulate high deformability of the cell. The cells were incubated in a microfluidic cavity (Figure 1C) filled with buffer solution that was retained during the characterization. The tips of the nanotweezers were introduced into the microfluidic slit, brought close to a cell that was trapped by a slight narrowing of the gap to 10 µm. Then a large actuation, (40 V DC actuation voltage) squeezed the cell to a reduced height of 4.4 µm. When the voltage was abruptly releases, the cell recovered its initial round shape with a dynamic that was captured by image processing. The transient of the shape recovery (Figure 5C) allows extracting the mechanical relaxation time of the fibroblast cell (43). This visco-elastic response proves to be a discrimination factor to detect cell infection and cell reaction to chemical treatment (38). Different tip topologies can be adapted according to the cell under stimulation. For example, to stimulate the fiber stress, sharp tips can be inserted into the cell at the fiber focal point and actuated.

9. ELECTRICAL CONDUCTION OF DNA, AND NANO-DOT COATED DNA IN AIR

Beyond biology, DNA molecules arouse multidisciplinary interests: in particular, the combination with electronics opens huge potentiality. Electronic device functionality can be enriched by the integration of remarkable biomolecular characteristics such as electrical hysteresis (44) or negative differential resistance (45). The electronic integration can also benefit from the unique capability of DNA to self-assemble in programmable structures (46) to realize 2D or 3D dense integration of nanometer sized devices (47). Also, the integration of biomolecules provides electronic components with natural optimized interfaces for bio sensing (48).

Nevertheless, the performances of these devices are lessened by the low electrical conductivity of DNA (49) and other biomolecules. Several methods have been proposed to circumvent this limitation such as DNA metallization (50) or nanodot incorporation (51), but the functionality of device requires an improved conductivity of the biomolecules while keeping their specific characteristic. For applications, several combinations of DNA dopants and ligands have to be evaluated. For this screening, SNT operation proves to be appropriate, the molecules can be easily trapped, manipulated and brought into different environments and solutions. The SOI technology provides the SNT with an excellent electrical isolation between its different parts. Thus the electrical characteristic of the sample bridging the 2 isolated tips can be precisely measured even it is highly resistive (up to the 10 TΩ range). This versatility is illustrated by a Pd nanodot binding protocol on DNA through thiocholine spacers, (Figure 6). DNA bundle is trapped with DEP according to the method of section 2, and rinsed in DI water during 30s to reduce the ionic charges of the bundle, a very low electrical conduction is indeed measured (R~2 TΩ). The bundle is immersed in the Pd-ND solution (52) for 1 min incubation at room temperature. Once retrieved from the solution, Pd-ND attachment gives a shiny image of the bundle (Figure 6C) and drastically increases its conductivity (R~1 GΩ). To ensure a stable conduction, clustered Pd-ND, not directly bound to the DNA were washed away by a final rinse in DI water and the electrical

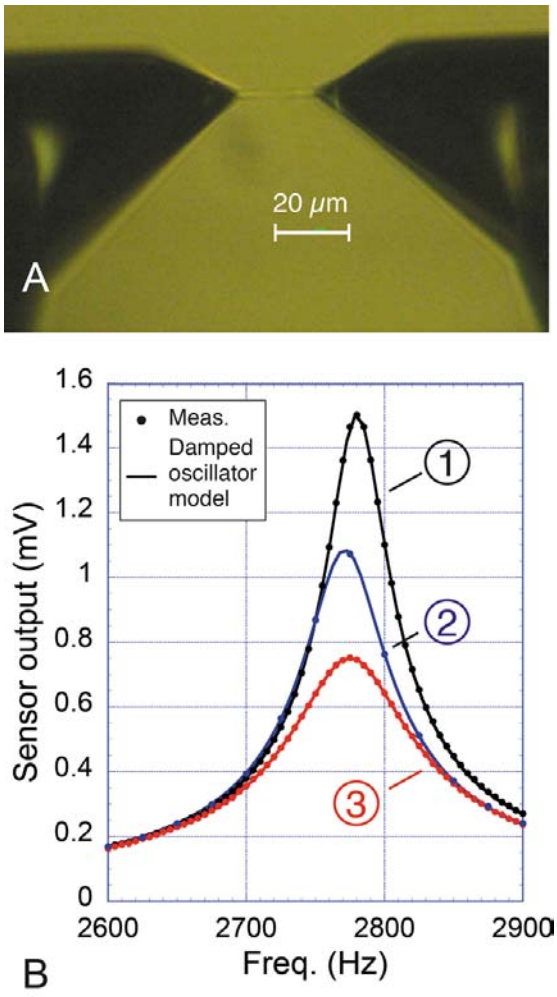

Figure 4. Characterization of gelatin fiber in solution. A: Photograph of the gelatin fiber retrieved from the solution as the final step of the process. B: Frequency responses of the tweezers during the trapping of gelatin. Curve 1: SNT in air. Curve 2: SNT in the gelatin solution before the trapping. Curve 3: SNT with the trapped gelatin in solution.

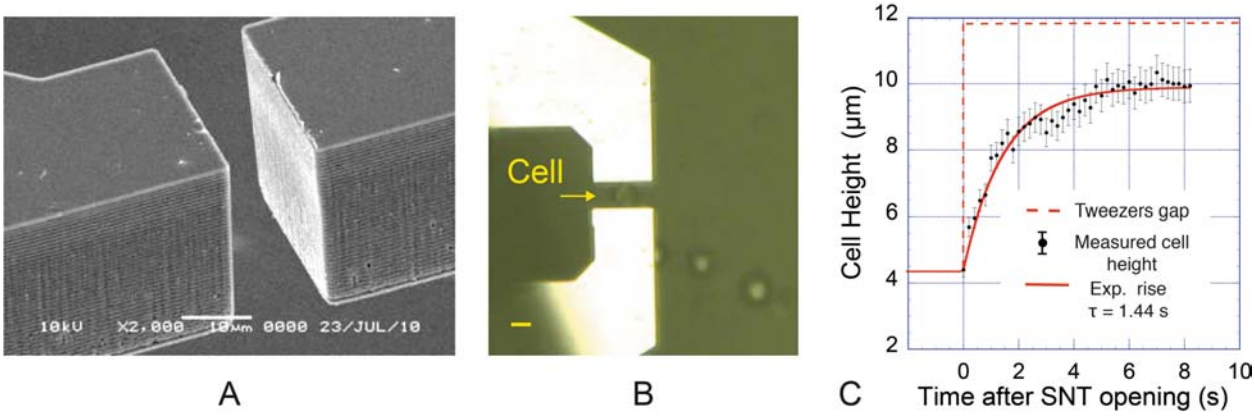

Figure 5.: Manipulation of cells with SNT. A: SEM picture of the flat tips used for cell trapping and compression, the distance between the two planes is 12 µm. The bar length is 10 µm. B: The SNT are introduced in the microfluidic cavity and a fibroblast cell is captured between the opposing tips whose gap was reduced to 10 µm by actuation (bar length). C: Subsequent larger actuation reduced the gap down to 4.4 µm and compressed the cell. After abrupt opening, the shape recovering of the fibroblast was recording by image processing. Evolution of the cell height with recovery time and best extraction with first order kinetic.

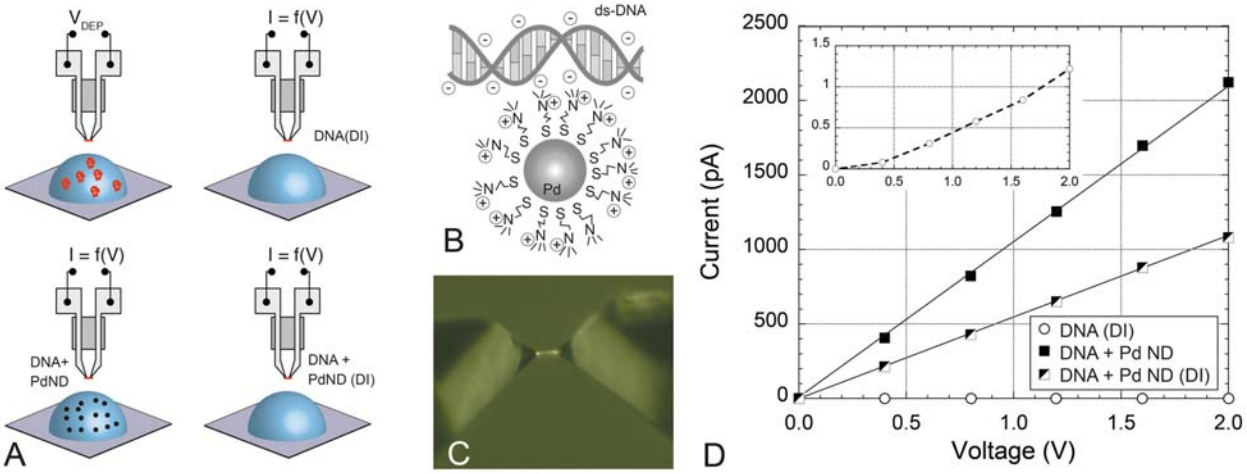

Figure 6. Experiment on PD-NP attachment on DNA bundle and conductance measurement. A: The DNA bundle is trapped by DEP and rinsed in DI water. The conductivity is measured: DNA(DI). DNA is incubated with PdNP and its conductivity is measured: DNA+ Pd NP. Final rinsing and measurement :DNA+Pd NP (DI). B: Structure of Pd NP attachment to DNA with charged thiocholine spacer. C: Visualization of DNA bundle after Pd NP attachment. D: Current voltage characteristics of the DNA bundle after rinsing, with Pd-NP and Pd-NP and rinsing. The insertion is an enlarged view of DNA(DI) curve. The SNT gap is 7µm and conductance measurement were performed at 20.5 °C and Rh 27%.

characteristic of the PD-ND-DNA bundle is repeatedly measured. The current-voltage measurements (Figure 6D) show an ohmic behavior with a resistance of 1.82 GΩ, the bundle conductivity has been increased by 3 orders of magnitude by the binding of the Pd-ND-TCB on DNA backbone.

The SNT provides additional capabilities by measuring the current passing through the wire during its deformation (elongation) by tips actuation. This piezo resistive sensing has revealed the volumetric ionic conduction of DNA bundle mediated by ambient humidity (30).

11. CONCLUDING NOTES

Inspired by the molecule manipulation instruments, such as atomic force microscope, we push our microsystem technology to propose the silicon nanotweezers, a miniature handy device capable of bio sensing at the molecular level in minute operations. The capabilities demonstrated so far encompass molecule trapping, mechanical characterization in air and in microfluidic cavity. Not only DNA but other filamentary molecules such as gelatin can be trapped between the actuated tips of the SNT. Molecular bundles can be manipulated and immersed in different solution for sensing or nano engineering as demonstrated by DNA fiber conductivity enhanced by gold nanoparticle attachment. Finally, the MEMS design provides the flexibility to customize the SNT to dedicated application. The tips shape can be adapted to the targeted samples, the initial gap and motion range can be optimized too, and tips surface can be functionalized for specific grafting.

Nevertheless, further improvements are actively underway to lower the SNT detection threshold down to the single molecular level (current detection is ~20 B-DNA molecules of 15 µm contour length). Several routes are under investigation combining mechanical design, signal processing by closed loop control and noise reduction. This progress are currently implemented and evaluated by real monitoring of various enzymatic reactions of trapped DNA and microtubules. As a MEMS, operated by the only electrical means, the SNT can harvest all modern electrical engineering progress to widen their application range. Automated bio sensing, electronic integration, parallel detection, autonomous operation and remote control can provide SNT with wide range of potential application. Thus, combining the current status of silicon nanotweezers achievement and the new improvements, SNT can be pushed to perform extremely sensitive bio detection though direct molecular interrogation for health and ecological concerns.

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Abbreviations: AC: Alternative Current; AFM: Atomic Force Microscope; DDL: Diffuse Double Layer, DEP: Di Electrophoresis; DNA: Deoxyribonucleic acid; MEMS: Micro Electro Mechanical Systems; Pd-ND: Pd Nanodot; SNT: Silicon nanotweezers; SOI: Silicon on Insulator;

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